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A New Genotype of TT Virus (TTV) Infection Among Colombian Native Indians

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Serum TTV DNA was assayed in 140 native Indians and 40 members of the general population in Colombia to determine the prevalence of TT virus (TTV) infection among Colombian native Indians. Of the 140 native Indians, 23 (16.4%) were positive for TTV DNA, compared to 4 (10.0%) of 40 from the general population (P = not significant). The prevalence of TTV DNA among native Indians was much higher than that of HBsAg and anti-HCV. Comparison of subjects with and without TTV DNA revealed no significant differences in all characteristics between the two groups. A phylogenetic tree, using the open reading frame 1 sequence (222 bp), indicated that the virus could be classified into four different genotypes, including three previously reported ones. The results show that TTV infection is common in Colombian native Indians without liver disease and also indicate the existence of a novel genotype of TTV. ***J. Med. Virol. 57:264–268, 1999.***

KEY WORDS: TT virus (TTV); Colombian native Indian; new genotype; molecular evolutionary analysis

INTRODUCTION

Recently, a new virus was isolated from the serum of a patient with posttransfusion hepatitis of unknown etiology, using representational difference analysis [Nishizawa et al., 1997]. This virus, designated as TT virus (TTV), is a single-stranded DNA virus [Okamoto et al., 1998]. TTV DNA was detected in 12% of Japanese healthy blood donors, although the serological prevalence of TTV infection in blood donors was lower than that in patients with fulminant or chronic cryptogenic liver diseases [Okamoto et al., 1998]. Recent studies, based on a phylogenetic tree constructed using the open reading frame (ORF) 1 sequence of TTV, showed that the virus could be classified into three different genotypes, namely, genotype 1 (G1), genotype 2 (G2), and genotype 3 (G3) [Simmonds et al., 1998]; however, the genotype-specific pathogenicity is unclear. There is little information on the prevalence of TTV infection in the world population in general and the origin and etiology of TTV are also unknown.

Among Colombian native Indians, a high prevalence of human T-cell lymphotropic virus-type I (HTLV-I), HTLV-II [Zaniovic et al., 1994; Fujiyoshi et al., 1995], and GB virus C (GBV-C) Asian type [Tanaka et al., 1998], which are mainly found in the Asian population, has been noted. A high prevalence of TTV is found in Japan [Okamoto et al., 1998]. We investigated the prevalence of TTV infection in three separate Colombian native Indian groups, the Inga, Kamsa, and Wayuu Indians, compared to the general population in Colombia. Determination of TTV DNA sequences in positive isolates, followed by molecular evolutionary analyses, revealed the existence of four different genotypes. Among Colombian native Indians, there exists a fourth novel genotype of TTV.

The nucleotide sequence data reported in this article will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the following accession numbers: AB016935–AB016961.

MATERIALS AND METHODS

Subjects

Forty-six serum samples were collected, after obtaining informed consent, from two different ethnic groups in the Andes highlands of Colombia (15 samples from Inga, 31 samples from Kamsa Indians), and 94 samples

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Fig. 1. Map of the three native Indian groups in Colombia.

were collected from another ethnic group on the Atlantic coast of Colombia (Wayuu Indians) (Fig. 1). As controls, 40 serum samples from the general population, including the Mestizo of mixed blood population but excluding native Indians, were also collected. These samples were taken in 1990 for the study of HTLV-I and -II, and HLA DRB1 and DQB1 haplotypes.

Detection of TTV DNA

Serum samples from all patients were stored at −80°C until assays. Serum DNA was extracted from 100-μl serum using proteinase K and sodium dodecyl sulfate (SDS) by the method described previously [Okamoto et al., 1990] and was determined with the Perkin-Elmer AmpliTaq Gold DNA Polymerase (Roche Molecular Systems, Branchburg, NJ) with seminested primers. The specific primers of TTV used for polymerase chain reaction (PCR) were kindly provided from Dr. Okamoto, Jichi Medical School, Tochigi, Japan [Okamoto et al., 1998]. In brief, the first round of PCR was performed with the sense primer NG059 and the antisense primer NG063 for 9 min at 96°C, followed by 35 cycles, consisting of denaturation for 30 sec at 94°C, annealing for 45 sec at 60°C, and extension for 45 sec at 72°C, using a 96-well cycler (GeneAmp 9600, Perkin-Elmer Cetus, Norwalk, CT). The second round of PCR was performed with the sense primer NG061 and the antisense primer NG063 for 25 cycles, under the same conditions as used for the first round of PCR.

The amplicons were analyzed by electrophoresis on 3% agarose gels, stained with ethidium bromide, and observed under ultraviolet light. The specificity of amplification was confirmed by direct sequencing of the amplified products with a 373A DNA Sequencer (Applied Biosystems, Foster City, CA).

Measurements of HBV- and HCV-Related Markers

All serum samples were assayed for hepatitis B surface antigen (HBsAg) (AUSRIA II, Abbott, Chicago, IL), antibody for hepatitis B surface antigen (anti-HBs) (Fujirebio, Tokyo, Japan), and antibody for hepatitis C virus (anti-HCV) (EIA-2, Ortho, Raritan, NJ).

Molecular Evolutionary Analysis

Molecular evolutionary analyses were performed to investigate the similarity between the isolates in this study and previously reported TTV strains from Japan and Europe [Nishizawa et al., 1997; Okamoto et al., 1998; Simmonds et al., 1998]. Using the computer program ODEN version 1.1.1 [Ina, 1994], the number of nucleotide substitutions per site and the genetic distances for all these isolates were estimated by the six-parameter method [Gojobori et al., 1982]. Based on these values, phylogenetic trees were constructed by both the neighbor-joining (N-J) method [Saitou et al., 1987], and unweighed pair grouping method with arithmetic means (UPGMA) [Nei, 1975]. To confirm the reliability of the phylogenetic tree, bootstrap resampling tests were performed 1,000 times [Felsenstein, 1985].

Statistical Analysis

Data were analyzed by Fisher's exact test, the Mann-Whitney U-test, Kruskal-Wallis test, or ANOVA. All tests of significance were two-tailed, with a P value of less than 0.05 considered to indicate statistical significance.

RESULTS

The mean ± SD age of the native Indians was 31.6 ± 14.2 years, their ages ranging from 8 to 80 years old, compared to 31.0 ± 14.7 years in the general population group. The male:female ratio was 41:99, compared to 12:28 (Table I). There were no significant differences in age and gender between these groups. There were also no significant differences among the three different Indian groups.

Of the 140 native Indians, 23 (16.4%) were positive for TTV DNA (2 [13.3%] of 15 Inga Indians, 3 [9.7%] of 31 Kamsa Indians, and 18 [19.1%] of 94 Wayuu Indians) compared to 4 (10.0%) of 40 in the control group. Although the prevalence of TTV infection tended to be higher among native Indians compared to the control group, the differences in prevalence between the two groups did not attain statistical significance. The prevalence of TTV infection was much higher than that of HBV and HCV infection in native Indians; of the 140 native Indians, none for HBsAg or anti-HCV. In regard to the profiles of TTV DNA-positive subjects, the mean ± SD age was 34.9 ± 15.0 years, the ages ranging from 14 to 74 years old, and the male:female ratio was 7:20. The alanine aminotransferase (ALT) concentrations of all of these subjects were normal. When the subjects were stratified into two groups, those with and without TTV DNA, no significant differences in the seroprevalence of HBsAg or anti-HCV, mean age, gender, or serum ALT concentrations were noted (Table II).

The nucleotide sequences obtained from all 27 iso-

TABLE I. Characteristics and Prevalence of TTV DNA Infection Among Colombians[a]

	Native Indians			Total	General population	*P*
	Inga	Kamsa	Wayuu			
Number	15	31	94	140	40	NS
Gender (M:F)	3:12	8:23	30:64	41:99	12:28	NS
Mean ± SD age (years)	35.4 ± 14.6	30.5 ± 12.3	31.5 ± 16.9	31.6 ± 14.2	31.0 ± 14.7	NS
HBsAg (+)	0	0	0	0	0	NS
Anti-HBs (+)	2	1	3	6 (4.3%)	1 (2.5%)	NS
Anti-HCV (+)	0	0	0	0	0	NS
TTV DNA (+)	2 (13.3%)	3 (9.7%)	18 (19.1%)	23 (16.4%)	4 (10.0%)	NS
G1	0	2	10	12	2	
G2	2	0	8	10	2	
G4	0	1	0	1	0	
ALT >25 (U/L)	0	2	2	4 (2.9%)	1 (2.5%)	NS

[a]Inga, Kamsa, and Wayuu are Colombian native Indian. NS = not significant; TTV = TT virus; HBsAg = hepatitis B surface antigen; anti-HBs = antibody against hepatitis B surface antigen; anti-HCV = antibody against hepatitis C virus; ALT = alanine aminotransferase. A *P* value was estimated between native Indians and the general population. G1 = genotype 1 (Group1); G2 = genotype 2; G4 = genotype 4. G1, G2, and G4 indicate genotypes of TTV.

TABLE II. Comparison of Subjects With and Without TTV DNA

	Positive for TTV DNA	Negative for TTV DNA	*P*
Number	27	153	
Gender [Male:Female]	7:20	46:107	NS
Age (years)	34.9 ± 15.0	31.0 ± 15.6	NS
HBsAg (+)	0 (0%)	0 (0%)	NS
Anti-HBs (+)	0 (0%)	7 (4.6%)	NS
Anti-HCV (+)	0 (0%)	0 (0%)	NS
ALT >25 (U/L)	0 (0%)	5 (3.3%)	NS

TTV = TT virus ; HBsAg = hepatitis B surface antigen; anti-HBs = antibody against hepatitis B surface antigen; anti-HCV = antibody against hepatitis C virus; ALT = alanine aminotransferase. A *P* value was estimated between native Indians and the general population. NS = not significant.

lates in this study were aligned with N22, TA278 (G1a), TX011 (G1b), TS003 (G2a), and NA004 (G2b) obtained from Japanese subjects [Okamoto et al., 1998] and three European isolates [Simmonds et al., 1998]. When a phylogenetic tree was constructed on the basis of a partial ORF 1 sequence (222 bp-excluded primer sequences), using two different strategies of molecular phylogenetic analysis by N-J method (Fig. 2A) and UPGMA (Fig. 2B), the 35 sequences were classified into four major genotypes. In brief, 17 isolates belonged to G1, 14 to G2, 3 obtained from European belonged to G3 [Simmonds et al., 1998], and 1 obtained from Kamsa Indians was assigned to a new genotype, which was tentatively named G4. On bootstrap analysis for evaluation of the statistical reliability of the tree, the G1, G2, G3, and G4 clusters exhibited 100%, 94.7%, 100%, and 100% reliability, respectively. The genetic distances between the three genotypes were very great, similar to those between different viruses, namely, 0.40844–0.68988 between G1 and G2, 0.39348–0.55145 between G1 and G3, 0.42815–0.50419 between G1 and G4, 0.24372–0.33907 between G2 and G3, 0.32908–0.46701 between G2 and G4, and 0.33433–0.36776 between G3 and G4. Such great genetic distances imply that the homology between the different genotypes was low, with 60.0%–69.0% between G1 and G2, 63.3%–70.5% between G1 and G3, 62.2%–68.6% between G1 and G4, 73.9%–80.1% between G2 and G3, 71.2%–73.4% between G2 and G4, and 71.8%–73.8% between G3 and G4.

In regard to the subtypes of TTV, according to the classification of TTV by Okamoto et al. [1998], 6 belonged to G1a, 11 to G1b, 2 to G2a, 2 to G2b, and 10 to G2 but not to G2a or G2b. As shown in Figure 2, we tentatively refer to these groups as G2c and G2d in this article. Interestingly, the proportion of subjects with the G2 in Colombia was higher than that in Japan, although there were no Colombian native Indian group-specific clusters (genotypes). Among the three different groups in this study, there were no significant differences in any characteristic.

DISCUSSION

A new single-stranded DNA virus designated TTV, which was isolated from the serum of a patient with posttransfusion hepatitis of unknown etiology, is often detected in patients with fulminant or chronic cryptogenic liver diseases [Okamoto et al., 1998]. However, this virus is also detected in healthy blood donors, and hence it remains unclear whether TTV is a direct cause of disease. In this study, we demonstrated that the prevalence of TTV infection among Colombian native Indians was high. Several additional points are also worthy of note. The prevalence of TTV infection among native Indians tended to be high, compared with that of the general Colombian population or that reported previously among Japanese blood donors [Okamoto et al., 1998], but no significant differences were found in the prevalence of TTV infection between the Colombian native Indians and the general Colombian population in this study. It was noteworthy that the prevalence of TTV single infection was higher than that of GBV-C single infection [Tanaka et al., 1998]. Furthermore, the serum ALT concentrations in all the 27 TTV DNA-positive subjects were normal. This indicated that most TTV DNA-positive subjects were carriers without obvious hepatitis, although this virus has been suggested

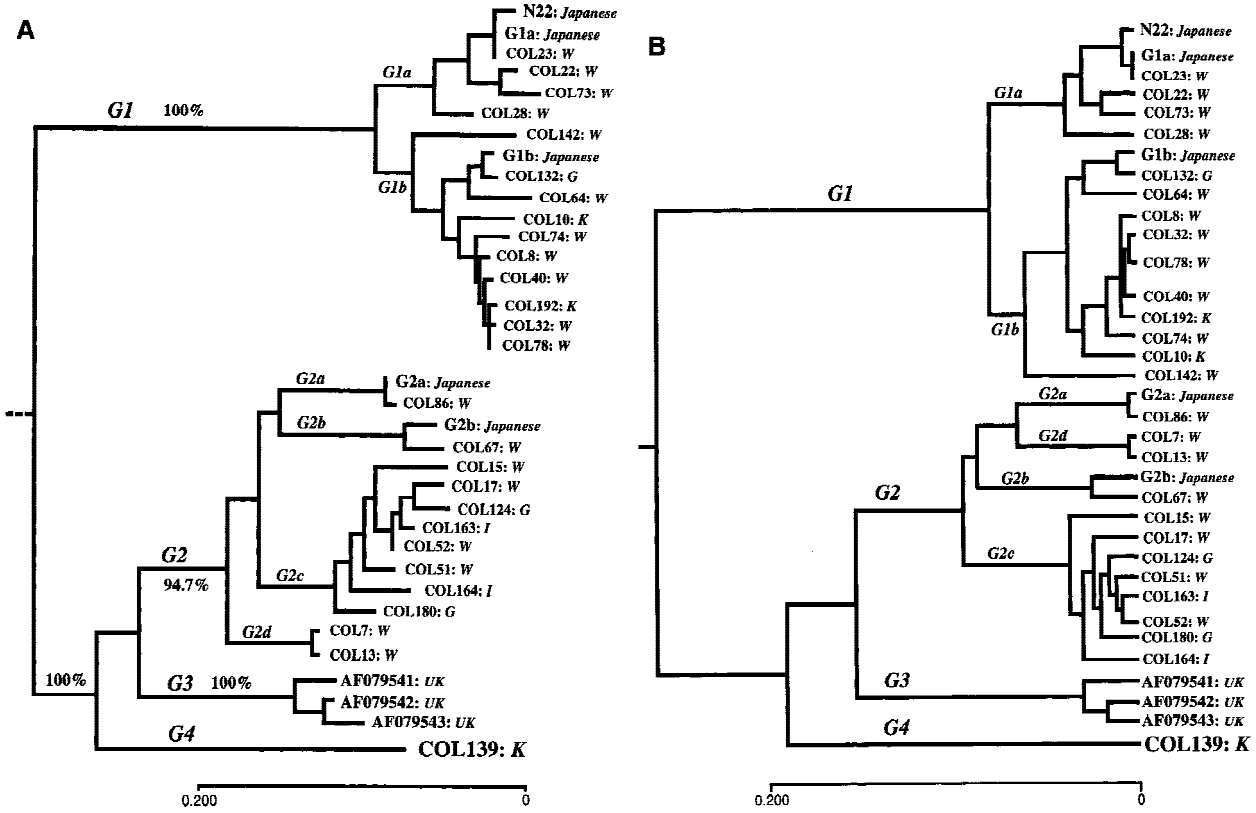

Fig. 2. Phylogenetic tree on the basis of the TT virus partial open reading frame 1 sequence as constructed by **(A)** the neighbor-joining (N-J) method, and **(B)** unweighed pair grouping method with arithmetic means (UPGMA). Note that there are four major clusters, tentatively named as genotype 1 (G1), genotype 2 (G2), genotype 3 (G3), and genotype 4 (G4). On bootstrap analysis for evaluation of the statistical reliability of the tree, the G1, G2, G3, and G4 clusters exhibited 100%, 94.7%, 100%, and 100% reliability, respectively. In regard to subtypes, according to the classification of TTV by Okamoto et al. [1998], 6 belonged to G1a, 11 to G1b, 2 to G2a, 2 to G2b, 8 to G2c, 2 to G2d, and 1 to G4 (named tentatively). N22, G1a, G1b, G2a, and G2b were obtained from Japan [Okamoto et al., 1998] and AF079541–AF079543 were obtained from the United Kingdom (UK) [Simmonds et al., 1998]. The horizontal bar indicates the number of nucleotide substitutions per site. COL = Colombian isolates, I = Inga Indians, K = Kamsa Indians, W = Wayuu Indians, G = general population.

as a cause of posttransfusion hepatitis [Okamoto et al., 1998].

Of the known animal single-stranded DNA viruses, human parvoviruses such as parvovirus B19 have been suspected repeatedly as the cause of non-A, non-B hepatitis in humans [Almeida et al., 1976; Appleton, 1977], although most do not cause hepatitis and have been able to coexist with humans for a long time without pathogenicity. There might still be specific genotypes of TTV causing severe liver diseases or the other diseases.

Another significant finding of this study was that of a new genotype of TTV in Kamsa Indians. That is to say, there existed one more, genotype 4 (G4), than the three previously reported genotypes of TTV [Simmonds et al., 1998]. The genetic distances between the four genotypes were as great as those between the genotypes of hepatitis C virus. Using two different strategies of molecular phylogenetic analysis by N-J method and UPGMA, four major clusters were obtained, and the high reliability of the four clusters was confirmed by bootstrap analysis. Moreover, the proportion of subjects with the genotype 2 (G2) in Colombia was higher than that in Japan, and two new subtypes of G2, namely G2c and G2d, were found, although there were no Colombian native Indian group-specific clusters. Further study will be required to define the clinical and virological characteristics of the G4 and the new G2 subtypes of TTV in this study. More interestingly, hepatitis virus markers such as HBsAg and anti-HCV were not found in TTV DNA-positive subjects, among whom only one was coinfected with GBV-C. These data indicate that TTV has been independently transmitted to the geographically isolated native Indians. The nature of TTV infection, including the epidemiology and routes of transmission, should be studied using the nucleotide sequences from all over the world. The exact nomenclature for these genotypes must await further discussion and a consensus between various research groups.

TTV DNA was previously reported more often in patients with fulminant or chronic liver diseases than in healthy blood donors [Okamoto et al., 1998]. However, it remains unclear whether TTV causes chronic liver diseases, and its natural course might be much like that of acute hepatitis, such as that caused by hepatitis A virus. In future experiments, it is necessary to analyze the prevalence of antibodies against TTV in order

to obtain more information about the natural course of TTV infection. Unfortunately, in this study there was little information on the routes of transmission, such as histories of transfusion or operation, use of intravenous drugs, and use of unsterilized syringes. To clarify more precisely the prevalence and clinical implications of TTV, further studies would be required on a large scale.

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REFERENCES

Almeida JD, Deinhardt F, Hoimes AW, Peterson DA, Wolfe L, Zuckerman AJ. 1976. Morphology of the GB hepatitis agent. Nature 210:283–291.

Appleton H. 1977. Virus particles in marmoset hepatitis. Nature 267: 729–730.

Felsenstein J. 1985. Evolution 39:783–791.

Fujiyoshi T, Yashiki S, Fujiyama C, Kuwayama M, Miyashita H, Ohnishi H, Blank M, Zaninovic V, Blank A, Cartier L, Byrnes J, Harrington W, Miura T, Hayami M, Tajima K, Sonoda S. 1995. Ethnic segregation of HTLV-I and HTLV-II carriers among South American native Indians. Intl J Cancer 63:510–515.

Gojobori T, Ishii K, Nei M. 1982. Estimation of average number of nucleotide substitutions when the rate of substitution varies with nucleotide. J Molec Evol 18:414–423.

Ina Y. 1994. ODEN: a program package for molecular evolutionary analysis and database search of DNA and amino acid sequences. Computer Applic Biosci 10:11–12.

Nei M. 1975. Molecular Population and Genetics. Amsterdam: North-Holland.

Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M. 1997. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. Biochem Biophys Res Comm 241:92–97.

Okamoto H, Yotsumoto S, Akahane Y, Yamanaka T, Miyazaki Y, Sugai Y, Tsuda F, Tanaka T, Miyakawa Y, Mayumi M. 1990. Hepatitis B viruses with precore region defects prevail in persistently infected hosts along with seroconversion to the antibody against e antigen. J Virol 64:1298–1303.

Okamoto H, Nishizawa T, Kato N,.Ukita M, Ikeda H, Iizuka H, Miyakawa Y, Mayumi M. 1998. Molecular cloning and characterization of a novel DNA virus (TTV) association with posttransfusion hepatitis of unknown etiology. Hepatol Res 10:1–16.

Saitou N, Nei M. 1987. The neighbor-joining method:a new method for reconstructing phylogenetic trees. Molec Biol Evol 4:406–425.

Simmonds P, Davidson F, Lycett C, Prescott LE, MacDonald DM, Ellender J, Yap PL, Ludlam CA, Haydon GH, Gillon J, Jarvis LM. 1998. Detection of a novel virus (TTV) in blood donors and blood products. Lancet 352:191–195.

Tanaka Y, Mizokami M, Orito E, Ohba K, Nakano T, Kato T, Kondo Y, Ueda R, Sonoda S, Tajima K, Miura T, Hayami M. 1998. GB virus C/hepatitis G virus infection among Colombian native Indians. Am J Tropic Med Hygiene 59(3):462–467.

Zaniovic V, Sanzon F, Lopez F, Velandia G, Blank A, Blank M, Fujiyama C, Yashiki S, Matsumoto D, Katahira Y, Miyashita H, Fujiyoshi T, Chan L, Sawada T, Miura T, Hayami M, Tajima K, Sonoda S. 1994. Geographic independence of HTLV-I and HTLV-II foci in the Andes Highland, the Atlantic Coast, the Orinoco of Colombia. AIDS Res Hum Retroviruses 10:97–101.